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Host-Derived ICAM-1 Glycoproteins Incorporated on Human Immunodeficiency Virus Type 1 Are Biologically Active and Enhance Viral Infectivity

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Human immunodeficiency virus type 1 (HIV-1) acquires several host cell membrane proteins when it buds from infected cells. To study the effect of virally incorporated host-derived ICAM-1 glycoproteins on the biology of HIV-1, we have developed a transient expression system that has enabled us to produce virus particles differing only in the absence or the presence of virion-bound ICAM-1. By using a single-round infection assay based on an ICAM-1-negative target T-cell line stably transfected with an HIV-1 long terminal repeat driven luciferase gene construct, we have been able to demonstrate that the acquisition of host-derived ICAM-1 by HIV-1 has functional significance, since it leads to a pronounced increase in viral infectivity (4.6- to 9.8-fold) in an ICAM-1/LFA-1-dependent fashion, as shown by blocking with anti-ICAM-1 and -LFA-1 antibodies. The same potentiating effect on viral infectivity was also observed with monocytoid cells. Studies of the kinetics of infection revealed that the positive effect mediated by virally embedded host cell membrane ICAM-1 is due to an increase in the efficiency of early steps in the viral life cycle. These results provide new insights into how incorporation of host proteins can modulate the biological properties of HIV-1. Our findings have direct clinical relevance, considering that ICAM-1 is expressed on the surface of virus-infected cells and, more importantly, that host-derived ICAM-1 has been shown to be acquired by clinical HIV-1 isolates grown on primary mononuclear cells. These data justify a more complete analysis of the other putative role(s) that virally incorporated ICAM-1 may play in the life cycle of HIV-1, for example, at the level of neutralization sensitivity.

The cell surface CD4 glycoprotein is a marker of auxiliary T lymphocytes, acts as a ligand for major histocompatibility complex class II (MHC-II) molecules, and provides a costimulatory signal necessary to achieve optimal cellular activation during antigen presentation (20, 21, 32, 36, 38, 39). CD4 is also recognized as the primary cellular receptor for the human immunodeficiency virus type 1 (HIV-1) (14, 35). The infection process of target cells with HIV-1 occurs via an interaction of the external membrane viral glycoprotein gp120 with the cell surface CD4 molecule, leading to membrane fusion (41). However, CD4 alone is not sufficient to allow merging of viral and cellular membranes as expression of human CD4 on murine cells fails to render them susceptible for virus infection or *env*-mediated syncytium formation (42). Recently, several studies have identified β-chemokine receptors as major fusion cofactors for T-cell- and macrophage-tropic HIV-1 isolates (3, 13, 15, 17, 18, 25). It has been postulated that chemokine receptors would interact with the CD4-gp120-gp41 complex, helping to expose the viral fusion peptide, which would then induce local attraction and destabilization of viral and cellular membranes, leading to fusion (16).

Nascent viral glycoproteins (gp120 and gp41) are preferentially incorporated into budding HIV-1 particles, while the bulk of host cell membrane glycoproteins are excluded from the assembling virus. Nevertheless, HIV-1 has been demonstrated to incorporate several foreign glycoproteins, such as different determinants of MHC-II (HLA-DR, -DP, and -DQ); β_2-microglobulin; CD43, CD44, CD55, CD59, CD63, and CD71; and adhesion receptors such as ICAM-1 and LFA-1 (4, 6, 9–11, 22, 26, 29, 31, 45–47, 51, 52). The incorporation of host-derived components appears to be selective, since not all cell surface molecules are found embedded within HIV-1. The observation that the receptor-linked transmembrane protein tyrosine phosphatase CD45 is not acquired by HIV-1 (47), even though it is the most abundant leukocyte cell surface glycoprotein (56), is clear evidence of the selectivity of this process. Such a selective incorporation phenomenon suggests that virally incorporated host molecules may play an important physiological role in the life cycle of HIV-1. This postulate is confirmed by the demonstration that virion-bound molecules can influence the biology of HIV-1 and its tropism (11). In addition, Saiffudin et al. demonstrated that CD55 and CD59, two GPI-linked complement control proteins, can protect HIV-1 from complement-mediated virolysis when incorporated into the budding virions (51).

A fair amount of information about the role of adhesion molecules in cell-to-cell transmission of HIV-1 is known. The presence of LFA-1 was found to be crucial for virus-mediated syncytium formation, and ICAM-1 was shown to be one of the receptors for this process (23, 28, 30, 48). However, our knowledge of the role played by virally incorporated host adhesion molecules in cell-free transmission of HIV-1 is more modest. One study has recently reported that cell-free virus produced from gamma interferon (IFN-γ)-treated U937 cells incorporated more host-derived ICAM-1 and HLA-DR molecules and possessed a greater infectivity toward CD4-negative cells (11). Thus, this study provides limited information on the putative biological functions of virally incorporated ICAM-1 in the biology of HIV-1.

Our goal was to determine if the acquisition of host-derived

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ICAM-1, by assembling HIV-1 particles, can modify virus infectivity and, if so, to identify the mechanism(s) responsible for modulation of HIV-1 biology. To achieve this task, we developed a transient expression system to produce highly concentrated viral particles differing only by the physical presence or absence of host cell membrane ICAM-1. We also developed an assay based on 1G5, a cell line stably carrying an HIV-1 long terminal repeat (LTR)-driven luciferase construct, that allows for highly sensitive and quantitative measurements of single-round viral infection events. With these tools, we were able to demonstrate that the presence of host-derived ICAM-1 on HIV-1 leads to a 4.6- to 9.8-fold enhancement in the process of virus infection. The observed phenomenon was not restricted to T cells, since it was also seen with monocytoid cells. Cross-linking experiments and studies conducted in the presence of an antiviral drug revealed that the enhancement of the infectious potential of the virus inoculum, which is mediated by the acquisition of host-derived ICAM-1, is attributable to an increase in the efficiency of the early steps of the HIV-1 replicative cycle.

MATERIALS AND METHODS

Cells. The 1G5 T-cell line, a Jurkat E6-1 derivative that harbors two stably integrated constructs made up of the luciferase gene under the control of the HIV-1_{SF2} LTR, was obtained from Estuardo Aguilar-Cordova and John Belmont through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH (2). BF24 is a THP-1 derivative that contains stably integrated copies of the HIV-1 LTR promoter linked to the reporter chloramphenicol acetyltransferase (CAT) gene (24, 53). This cell line was obtained from Barbara K. Felber and George N. Pavlakis through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH. These cells were maintained in complete culture medium made of RPMI-1640 supplemented with 10% fetal bovine serum (FBS; Hyclone Laboratories, Logan, Utah), glutamine (2 mM), penicillin G (100 U/ml), and streptomycin (100 μg/ml). In our studies, we have also used human embryonic kidney 293T cells, which express the simian virus 40 large-T antigen (49). These cells were maintained in Dulbecco modified Eagle medium (DMEM) supplemented with 10% FBS, L-glutamine (2 mM), penicillin G (100 U/ml), and streptomycin (100 μg/ml). This cell line was kindly provided by Warner C. Greene (J. Gladstone Institutes, San Francisco, Calif.).

Antibodies. The anti-ICAM-1 antibody (anti-CD54) RR1/1.1.1 was kindly provided by Robert Rothlein (Boehringer Ingelheim, Ridgefield, Conn.) (50). TS1/18.1 (anti-CD18), TS1/22.1 (anti-CD11a), and OKT3 (anti-CD3) hybridomas were obtained from the American Type Culture Collection (Rockville, Md.). Hybridomas that produce 31-90-25, an antibody recognizing the HIV-1 major viral core protein p24, and SIM.4, an anti-CD4 antibody, were also used for our studies. Both hybridomas were supplied by the AIDS Research and Reference Reagent Program, Division of AIDS, National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH). Antibodies from these producer cell lines were purified with the mAbTrap protein G affinity column according to the instructions of the manufacturer (Pharmacia LKB Biotechnology AB, Uppsala, Sweden). NKI-L16 (anti-CD11a) was obtained from Carl C. Figdor (University Hospital Nijmegen, Nijmegen, The Netherlands) (34), while MEM83 and MEM30 (anti-CD11a) were kind gifts from Vaclav Horejsi (Institute of Molecular Genetics, Prague, Czech Republic) (5). Unconjugated goat anti-mouse immunoglobulin G (IgG) and R-phycoerythrin-conjugated goat anti-mouse IgG were purchased from Pel-Freez (Rogers, Ark.) and Caltag Laboratories (San Francisco, Calif.), respectively. 31-90-25 was biotinylated with NHS-LC-Biotin (Pierce, Rockford, Ill.).

Plasmids. pNL4-3 is a full-length infectious molecular clone of HIV-1 (1). This vector was provided by the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH. pCD1.8 is a eukaryotic expression vector containing the entire human ICAM-1 cDNA and was obtained from Timothy A. Springer (Center for Blood Research, Boston, Mass.) (55).

Production of virus stocks. Virus stocks were produced by $CaPO_4$ transfection of the infectious molecular clone pNL4-3 with or without the ICAM-1 encoding plasmid pCD1.8 by a modification of the method of Chen and Okayama (12). Briefly, 293T cells were plated 24 h before transfection at a concentration of 5×10^5 cells per 3 ml of DMEM into each well of 6-well plates. All solutions were brought to room temperature before use. Immediately before transfection, DNA (10 μg of each plasmid) was added to 25 μl of 2.5 M $CaCl_2$ and the volume was completed to 250 μl with distilled water. This solution was then added drop by drop to 250 μl of 2× HEPES-buffered saline (HBS) solution (280 mM NaCl, 50 mM HEPES, 1.5 mM Na_2HPO_4 [pH 7.05]), and the resulting mixture was stored at room temperature for 5 min. This DNA-HBS mixture was finally added drop by drop to plated 293T cells before incubation at 37°C under a 5% CO_2 atmosphere. At 16 h after transfection, the cells were washed twice with 3 ml of phosphate-buffered saline (PBS) and were incubated for an additional 24 h with 3 ml of DMEM supplemented with 10% FBS. Virion-containing supernatants were filtered through a 0.45-μm-pore-size cellulose acetate membrane (Millipore, Bedford, Mass.), were aliquoted in 200-μl fractions, and were finally frozen at −85°C until needed. Virus stocks were normalized for virion content by a commercial assay for the viral major core protein p24 (Organon Teknika, Durham, N.C.). The percentage of pelletable p24 protein was assessed following ultracentrifugation of virus preparations (Heraeus model Contifuge 28RS; 16,000 × *g* for 90 min at 4°C). All virus stocks underwent one freeze-thaw cycle prior to initiation of infection studies. Cotransfection of pNL4-3 with pCD1.8 led to the production of virus stocks called ICAM-1/POS (because such virions bear host-derived ICAM-1). Transfection of 293T cells with pNL4-3 only resulted in the production of virus preparations named ICAM-1/NEG, since cellular ICAM-1 glycoproteins were not found embedded within such virions.

Flow cytometric analysis of transfected cells. An aliquot of $CaPO_4$-transfected 293T cells (5×10^5) was washed twice with PBS containing 2% FBS (washing medium). Pelleted cells were incubated for 30 min on ice with a saturating concentration of RR1/1.1.1 (1 μg/10^6 cells), an anti-ICAM-1 antibody. The cells were washed twice with washing medium and incubated with a saturating concentration of R-phycoerythrin-conjugated goat anti-mouse IgG for 30 min on ice. Finally, cells were washed twice in PBS and were resuspended in 300 μl of PBS containing 1% (wt/vol) paraformaldehyde prior to flow cytometry analysis (EPICS XL; Coulter Corporation, Miami, Fla.). Controls consisted of commercial isotype-matched murine monoclonal antibodies (Sigma, St. Louis, Mo.). Intracellular viral p24 staining of transiently transfected 293T cells was performed with a commercial intracellular flow cytometry kit (Fix & Perm cell permeabilization kit from Caltag Laboratories) in accordance with the supplier's instructions. Intracellular flow cytometry was carried out with a combination made of biotinylated 31-90-25 and R-phycoerythrin-conjugated Streptavidin (Jackson ImmunoResearch Laboratories, Inc., West Grove, Pa.).

Detection of virally incorporated ICAM-1. Virally embedded ICAM-1 was monitored by immunocapture with magnetic beads as described previously (9). In brief, 12.5×10^6 magnetic beads (BioMag, Fc specific; PerSeptive Diagnostics, Inc., Cambridge, Mass.), previously coated with the anti-ICAM-1 antibody RR1/1.1.1, were incubated with similar amounts of HIV-1_{NL4-3} ICAM-1/NEG and HIV-1_{NL4-3} ICAM-1/POS stocks standardized in terms of the viral core p24 protein (5,000 pg of p24) in a final volume of 1 ml of binding medium (PBS plus 0.1% bovine serum albumin). This mixture was incubated for 1 h at 4°C on a rotating plate. The beads were washed five times in binding medium with a magnetic separation unit and were resuspended in 100 μl of binding medium. The amount of immunocaptured HIV-1 particles was assessed by measuring viral p24 protein content found associated with immunomagnetic beads. Magnetic beads coated with anti-CD3 antibodies (clone OKT3) were used as a negative control.

Stimulation and HIV-1 infection of 1G5 and BF24 cells. 1G5 cells either were left untreated or were stimulated with 3 μg of phytohemagglutinin-P (PHA-P; Sigma) per 10^6 cells for 48 h at 37°C. In parallel, 1G5 cells were also inoculated with HIV-1_{NL4-3} stocks bearing or not bearing ICAM-1 molecules (ICAM-1/POS and ICAM-1/NEG, respectively). For virus infection, stocks of HIV-1, standardized in terms of p24 content (10 ng of p24), were first incubated in the presence or absence of RR1/1.1.1 at a concentration of 20 μg/ml for 30 min at 37°C. This concentration has been shown to be sufficient to block cell-to-cell adhesion which is dependent on the ICAM-1–LFA-1 interaction (43). For some experiments, 1G5 cells were first incubated for 30 min at 37°C with MEM30, an anti-CD11a antibody that blocks ICAM-1–LFA-1 interaction, at a concentration of 10 μg/ml before viral infection. Thereafter, the virus-antibody mixture was incubated with 1G5 cells (10^5) and the infection was allowed to proceed for another 90 min at 37°C. The cells were then washed twice with PBS, resuspended in 200 μl of RPMI complete medium, and were transferred to a 96-well flat-bottom tissue culture plate (Microtest III, Falcon; Becton Dickinson, Lincoln Park, N.J.). After a 24-h incubation, a combination of 1 μM azidothymidine (3′-azido-2′,3′-dideoxythimidine [AZT]) and 30 μM didanosine (2′,3′-dideoxyinosine [ddI]) was added to abrogate any reinfection events. Both antiviral drugs were obtained from Sigma. Finally, HIV-1-infected cells were incubated for an additional period of 24 h at 37°C.

Luciferase activity in untreated, PHA-P-stimulated, and HIV-1-infected 1G5 cells was monitored as described previously (8). In brief, 100 μl of cell-free supernatant was discarded from each well, and 25 μl of 5× cell culture lysis buffer (125 mM Tris phosphate [pH 7.8], 10 mM dithiothreitol [DTT], 5% Triton X-100, 50% glycerol) was added to the wells before incubation at room temperature for 30 min. An aliquot of cell lysate (20 μl) was mixed with 100 μl of luciferase assay buffer [20 mM tricine, 1.07 mM $(MgCO_3)_4 \cdot Mg(OH)_2 \cdot 5H_2O$, 2.67 mM $MgSO_4$, 0.1 mM EDTA, 270 μM coenzyme A, 470 μM luciferin, 530 μM ATP, 33.3 mM DTT]. The sample was introduced into the counting chamber of a standard liquid scintillation counter equipped with a single-photon monitor software (Beckman Instruments, Fullerton, Calif.). Total photon events were measured over 30 s. In some assays, inhibition of HIV-1 infection with AZT was achieved as follows. Cells (10^5) were preincubated for 60 min at 37°C with increasing concentrations of AZT. Then, the cells were washed twice with PBS and virus infection was continued as described above in complete culture medium containing the indicated concentrations of AZT.

BF24 cells were also infected with HIV-1_{NL4-3} stocks bearing or not bearing

host-derived ICAM-1 molecules (ICAM-1/POS and ICAM-1/NEG, respectively) under conditions similar to those with 1G5 cells, with slight modifications. Briefly, virus stocks were incubated in the presence or absence of RR1/1.1.1 at 20 μg/ml for 30 min at 37°C. Afterward, a fixed amount of each virus stock (25 ng of p24) was added to BF24 (5×10^5) cells, and the infection was monitored for 90 min at 37°C. The cells were then washed twice with PBS, resuspended in 1 ml of complete culture medium, and transferred to a 24-well flat-bottom tissue culture plate. After a 24-h incubation at 37°C, 1 μM AZT and 30 μM ddI were added. Finally, cells were lysed 24 h later and CAT production was monitored with a commercial enzyme-linked immunosorbent assay (ELISA) kit in accordance with the instructions of the supplier (Boehringer GmbH, Mannheim, Germany).

Kinetics of virus infection. The kinetics of infection of 1G5 cells with HIV-1_{NL4-3} stocks bearing or not bearing host-derived ICAM-1 (ICAM-1/POS and ICAM-1/NEG, respectively) were determined as follows. 1G5 cells (10^5) were inoculated with each virus preparation (10 ng of p24). At specific time points after virus infection, plasma-pooled purified human IgG from HIV-1-infected subjects was added to each well at a final concentration of 200 μg/ml to stop the infection process. The cells were washed twice with PBS, resuspended in 200 μl of complete culture medium, and transferred to a 96-well plate. After a 24-h incubation, 1 μM AZT and 30 μM ddI were added. Finally, 24 h later, the cells were lysed and luciferase activity was evaluated.

Cross-linking experiments. 1G5 cells were first incubated for 30 min on ice with anti-LFA-1 alone or with anti-LFA-1 and anti-CD4 antibodies (0.1 μg of each per 10^5 cells). These studies were performed with several anti-LFA-1 antibodies (anti-CD18 for TS1/18.1, anti-CD11a for TS1/22.1, anti-CD18 for NKI-L16, and anti-CD11a for MEM83) and one anti-CD4 antibody (SIM.4). Then, the cells were washed twice with PBS and were incubated at 37°C for 48 h in 96-well flat-bottom tissue culture plates (Microtest III, Falcon) that were first coated with goat anti-mouse IgG (GAM) as follows. The plates were incubated with 100 μl of GAM per well at a concentration of 10 μg/ml in coating buffer (50 mM Tris-HCl [pH 9.5]) for 24 h at 4°C. The plates were washed twice with 200 μl of PBS, and unoccupied sites were blocked by incubation at 37°C for 30 min with 100 μl of PBS per well containing 5% FBS.

RESULTS

Elaboration of a transient expression system. To evaluate the putative role played by a specific virally incorporated host cell membrane protein on the biology of HIV-1, one has to develop a system that leads to the production of progeny viruses differing exclusively in the physical presence of the selected molecule on the produced virions. The 293T human embryonic kidney cells, which are highly transfectable by the calcium phosphate method and are negative for ICAM-1 surface expression, can yield high-titer viral stocks relatively free of cellular debris (data not shown). Expression of surface ICAM-1 and intracellular viral p24 was monitored in 293T cells cotransfected with pNL4-3, an infectious molecular clone of HIV-1, and pCD1.8, a eukaryotic expression vector encoding for human ICAM-1. Flow cytometry analysis revealed that close to 100% of the cotransfected cells express high levels of ICAM-1 on their surface (Fig. 1A) and that almost 90% are positive for intracellular viral p24 protein (Fig. 1B). The results from this set of experiments suggest that all virus-producing cells will express surface ICAM-1. Therefore, progeny virions budding from cotransfected 293T cells will be shed from ICAM-1-positive cells.

Evaluation of the presence of virion-bound host cell membrane ICAM-1. The efficient expression of high levels of ICAM-1 on the surface of virally expressing 293T cells does not necessarily signify that this glycoprotein will be efficiently incorporated within nascent virus particles. We thus monitored the acquisition of host-derived ICAM-1 by HIV-1_{NL4-3} particles budding from cotransfected 293T cells by immunocapture with magnetic beads, as described previously (9). Background levels of ICAM-1-free virions (ICAM-1/NEG), which were harvested from 293T cells transfected with pNL4-3 only, were captured by magnetic beads coated with anti-CD3 (clone OKT3) and anti-ICAM-1 (clone RR1/1.1.1) antibodies (Table 1). ICAM-1/POS particles, which were produced by 293T cells cotransfected with pNL4-3 and pCD1.8, were also not efficiently captured by magnetic beads coated with anti-CD3.

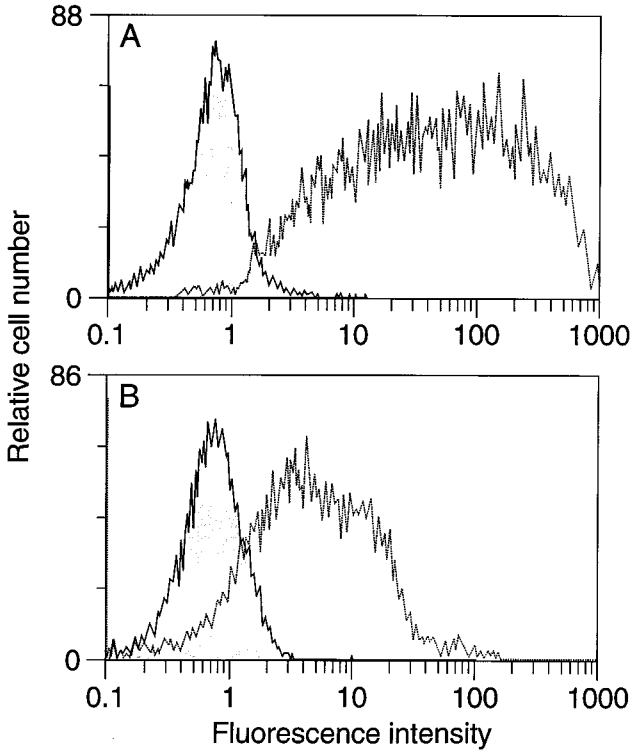

FIG. 1. FACS analysis of 293T cells cotransfected with pCD1.8 and pNL4-3. (A) At 40 h after transfection, 293T cells were harvested and labelled with an anti-ICAM-1 monoclonal antibody (clone RR1/1.1.1 [empty profile]) or an isotype-matched commercial control antibody (shadowed profile) followed by an R-phycoerythrin-conjugated GAM for the detection of cell surface ICAM-1 expression. (B) Cells were fixed, permeabilized, and labelled with a combination made of biotinylated anti-p24 antibodies (clone 31-90-25 [empty profile]) or an isotype-matched commercial control antibody (shadowed profile) and R-phycoerythrin-conjugated Streptavidin for the detection of intracellular viral p24 protein expression.

However, antibodies against ICAM-1 efficiently captured virions harvested from 293T cells cotransfected with pNL4-3 and pCD1.8 (ICAM-1/POS). These data indicate that host-derived ICAM-1 glycoproteins are found embedded on virus particles produced by ICAM-1-positive 293T cells. Therefore, the present transient expression system is appropriate to achieve the production of HIV-1 particles bearing or not bearing virally acquired cellular ICAM-1.

TABLE 1. Immunomagnetic capture of HIV-1 particles produced by transfected 293T cells[a]

Virus stock	Capture of HIV-1 with magnetic beads coated with[b]:	
	anti-CD3 (OKT3)	anti-ICAM-1 (RR1/1.1.1)
ICAM-1/NEG	19.5 ± 0	39.0 ± 3.0
ICAM-1/POS	13.5 ± 3.0	757.5 ± 45.0

[a] HIV-1_{NL4-3} ICAM-1/NEG or HIV-1_{NL4-3} ICAM-1/POS particles (5,000 pg of p24) were incubated with 12.5×10^6 antibody-coated magnetic beads for 1 h at 4°C on a rotating plate. After extensive washing, the amounts of immunocaptured HIV-1 particles were assessed by measuring viral p24 protein content found associated with immunomagnetic beads.

[b] Values are picograms of captured p24. Results are the means ± SEM for triplicate samples.

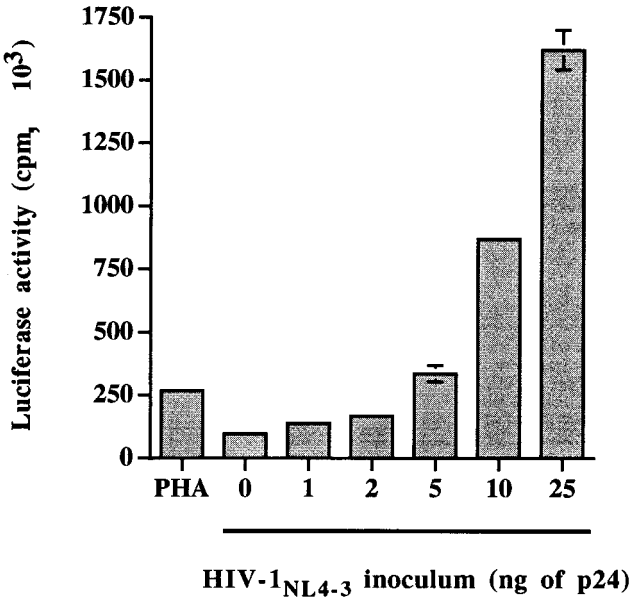

FIG. 2. Dose-dependent increase of HIV-1 LTR-driven luciferase activity after infection with increasing concentrations of HIV-1. 1G5 cells were infected with increasing amounts of ICAM-1-free HIV-1 particles. After 24 h of infection, 1 μM AZT and 30 μM ddI were added and, 24 h later, cells were lysed before monitoring luciferase activity. In this experiment, PHA-P was used as a positive control for HIV-1 LTR-mediated transcriptional activation of the luciferase reporter gene. Result are the means ± standard errors of the means (SEM) for triplicate samples.

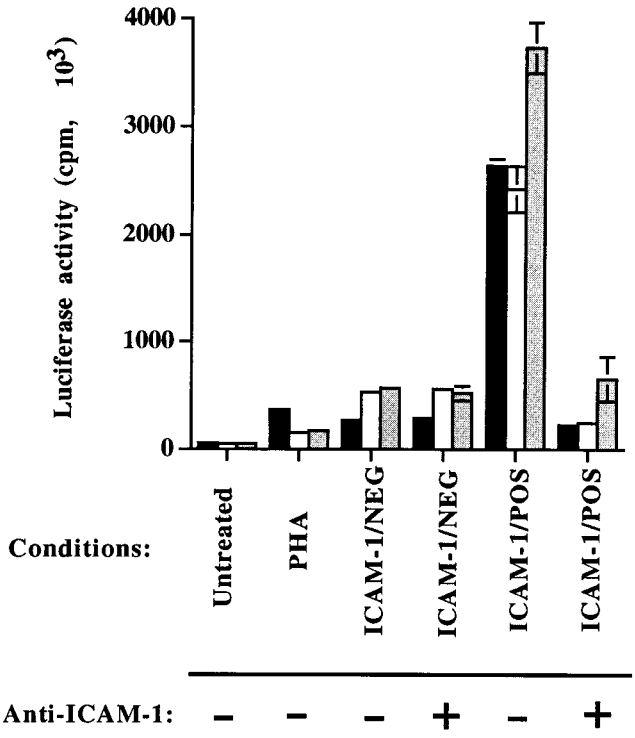

FIG. 3. The presence of host-derived ICAM-1 leads to an enhancement of virus-induced HIV-1 LTR-driven luciferase activity. 1G5 cells were infected with 10 ng of p24 from ICAM-1-free virions (ICAM-1/NEG) or ICAM-1-bearing progeny viruses (ICAM-1/POS) produced by three different independent transfections (black boxes, stock A; white boxes, stock B; shadowed boxes, stock C). Virions were first pretreated or untreated with 20 μg of RR1/1.1.1 (anti-ICAM-1 antibody) per ml. At 24 h postinfection, 1 μM AZT and 30 μM ddI were added and, 24 h later, cells were lysed before monitoring of luciferase activity. Data are the means ± SEM for triplicate samples.

Standardization of a sensitive system to quantitatively evaluate single-round infection process. The biological role played by virally acquired host cell membrane ICAM-1 proteins was then investigated, since we believe that the presence of host-derived ICAM-1 may modulate the biological characteristics of HIV-1. This postulate is based on the assumption that virally embedded ICAM-1 would interact with its counterreceptor LFA-1 on the target cell. An assay allowing a sensitive and quantitative determination of single-round infection events was then used. This test relies on 1G5, an ICAM-1-negative cell line expressing moderate levels of both surface LFA-1 and CD4 molecules (data not shown). These cells contain two integrated copies of the HIV-1_{SF2} LTR promoter driving the expression of the luciferase reporter gene. Such a cell line has been reported to possess high responsiveness to the viral transactivating protein Tat (2) and is thus an ideal tool to measure the extent of virus infection. First, we were interested in monitoring the sensitivity of 1G5 cells to infection using different amounts of virus particles. For this purpose, 1G5 cells were inoculated with increasing concentrations of HIV-1_{NL4-3} ICAM-1/NEG (1, 2, 5, 10, and 25 ng of p24). As shown in Fig. 2, luciferase activity monitored after 48 h of infection was found to be directly proportional and linear to the quantity of viruses added at the time of initial virus infection. The standard deviations were minimal for triplicate samples (less than 5%), and the levels luciferase activity in infected cells were well above background levels found in uninfected cells.

Virally incorporated host cell membrane ICAM-1 can upregulate HIV-1 infectivity. The functionality of host-acquired ICAM-1 molecules was then evaluated by infecting 1G5 cells with similar amounts of ICAM-1/NEG and ICAM-1/POS virions. The normalization of virus stocks is a determining factor when the ultimate goal is to quantitatively assess the process of virus infection. Our virus preparations were standardized in term of p24 contents based on the fact that similar contents of pelletable p24 protein (>90%) were found for virus stocks harvested from either transfected (pNL4-3) or cotransfected (pNL4-3 and pCD1.8) 293T cells (data not shown). Results from Fig. 3 indicate that the presence of host-derived ICAM-1 glycoproteins on HIV-1 particles is associated with a 4.6- to 9.8-fold increase of HIV-1 LTR-mediated reporter gene activity in 1G5 cells. These experiments were carried out with three different virus stocks originating from independent transfections, suggesting that the marked enhancement of HIV-1 infectivity, which is conferred by the acquisition of host-derived ICAM-1 by HIV-1, is not an epiphenomenon. It should be noted that infection was performed with an inoculum of HIV-1 particles (10 ng of p24) that is found in the linear part of the dose-response curve of virus infection (Fig. 2). Levels of luciferase activity following infection with ICAM-1-bearing particles pretreated with an anti-ICAM-1 antibody (clone RR1/1.1.1) returned to the levels of luciferase activity obtained after infection with ICAM-1-free virions. These experiments were conducted with the monoclonal antibody RR1/1.1.1 at 20 μg/ml, a concentration known to be sufficient to block cell-to-cell adhesion dependent on the ICAM-1/LFA-1 interaction (43). The blockade effect mediated by the anti-ICAM-1 antibody is not due to steric hindrance, since luciferase activity seen in the presence of RR1/1.1.1 was returned to levels of reporter gene activity detected in 1G5 cells infected with progeny virus devoid of host-derived ICAM-1.

The active participation of virally incorporated host ICAM-1

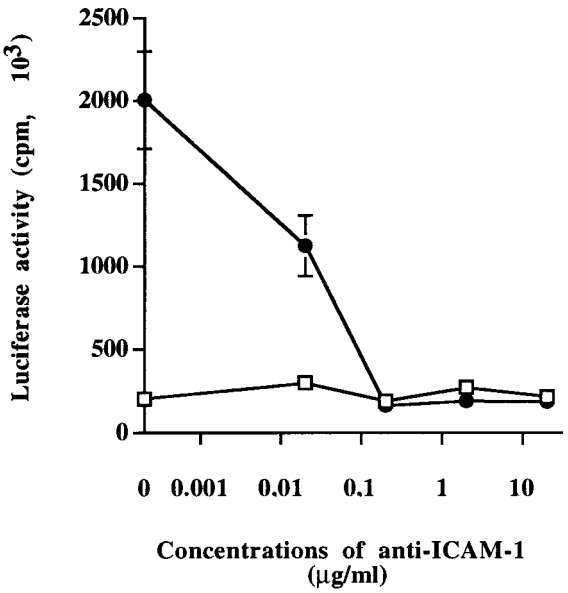

FIG. 4. Dose-dependent inhibition of the enhancement of virus-induced HIV-1 LTR-driven luciferase activity with RR1/1.1.1. 1G5 cells were infected with 10 ng of p24 from ICAM-1-bearing virions (ICAM-1/POS [●]) or ICAM-1-free progeny viruses (ICAM-1/NEG [□]), which were pretreated or untreated with different concentrations of RR1/1.1.1 (anti-ICAM-1 antibody). At 24 h postinfection, 1 μM AZT and 30 μM ddI were added and, 24 h later, cells were lysed before monitoring of luciferase activity. Results are the means ± SEM for samples performed in triplicate.

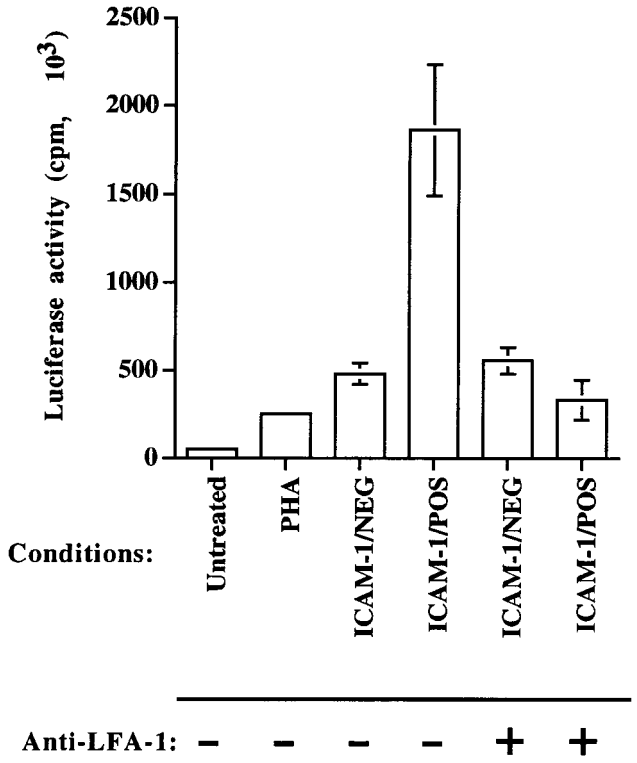

FIG. 5. Interaction between virally acquired host ICAM-1 and cell surface LFA-1 is responsible for the enhancement of HIV-1 infectivity. 1G5 cells were treated with MEM30 (anti-CD11a antibody [10 μg/ml]) prior to inoculation with 10 ng of p24 from ICAM-1-bearing virions or ICAM-1-free progeny viruses. At 24 h postinfection, 1 μM AZT and 30 μM ddI were added, and, 24 h later, cells were lysed before monitoring of luciferase activity. Results are the means ± SEM for samples performed in triplicate.

molecule in the increase of HIV-1 LTR-driven gene expression was further studied by performing similar infection studies in the presence of increasing concentrations of anti-ICAM-1 antibodies. In this experiment, virus stocks were treated with increasing concentrations of RR1/1.1.1 (0.002, 0.02, 0.2, 2, and 20 μg/ml) prior to HIV-1 infection. From Fig. 4, two important observations can be made. First, for untreated virus stocks, a 10-fold increase in virus infectivity was conferred by virion-bound host-derived ICAM-1 glycoproteins. Second, increasing concentrations of RR1/1.1.1 gradually abrogated the infectivity advantage associated with the incorporation of cellular ICAM-1. Data from this set of experiments are another indication that the decrease of virus infectivity mediated by RR1/1.1.1 is not due to steric hindrance.

A further evidence of the importance of the interaction between virion-bound ICAM-1 and cell surface LFA-1 in the process of virus infection came from the demonstration that preincubation of 1G5 cells with MEM30, an anti-LFA-1 antibody (anti-CD11a) known to abolish binding of ICAM-1 to LFA-1, also led to the disappearance of the increase in HIV-1 infectivity (Fig. 5).

Enhancement of HIV-1 LTR-driven reporter gene expression following infection of 1G5 cells with ICAM-1-bearing virions is independent of signal transduction events. In order to confirm that the marked increase in HIV-1 LTR-dependent luciferase activity was due to an enhancement of virus infectivity, cells were pretreated with increasing concentrations of AZT prior to virus inoculation. This experiment was designed to block the initial infection of 1G5 cells and to allow detection of any signaling events, mediated by the binding of the virus to its target cell, that may result in up-regulation of HIV-1 LTR-dependent reporter gene activity. A dose-dependent inhibition of HIV-1 LTR-mediated luciferase activity was seen following infection of 1G5 cells with virions bearing or not bearing host-derived ICAM-1 in the presence of increasing concentrations of AZT (0.005, 0.05, 0.5, and 5 μM) (Fig. 6). More importantly, a similar percentage of inhibition was detected when cells were pretreated with 5 μM AZT prior to inoculation with ICAM-1/NEG (71% inhibition) or ICAM-1/POS (74% inhibition) virions. It can be postulated from these data that the enhancement of HIV-1 LTR-driven luciferase activity following infection with viruses bearing host cell membrane ICAM-1 is not mediated by signal transduction pathways.

To further substantiate this fact, we performed cross-linking experiments using antibodies specific for cell surface molecules that would be multimerized on 1G5 cells following the binding of ICAM-1-bearing virions. The cells were first incubated with saturating concentrations of several anti-LFA-1 antibodies alone (TS1/18.1, TS1/22.1, NKI-L16, and MEM83) or in combination with SIM.4, an anti-CD4 antibody specific for the gp120 binding site (44). These cells were then incubated in plates precoated with goat anti-mouse antibodies to achieve multimerization of LFA-1 alone or LFA-1 and CD4. None of the combinations of antibodies used were able to trigger the activation of HIV-1 LTR-dependent luciferase activity in 1G5 cells (Fig. 7). Thus, it eliminates the possibility that the marked increase in HIV-1 LTR-dependent reporter gene activity after inoculation with virus particles bearing host-derived ICAM-1 is due to signal transduction.

Virally incorporated host cell membrane ICAM-1 glycoproteins increase and accelerate the rate and efficiency of virus entry and infection. We were interested in identifying the mechanism(s) by which the presence of host-derived ICAM-1

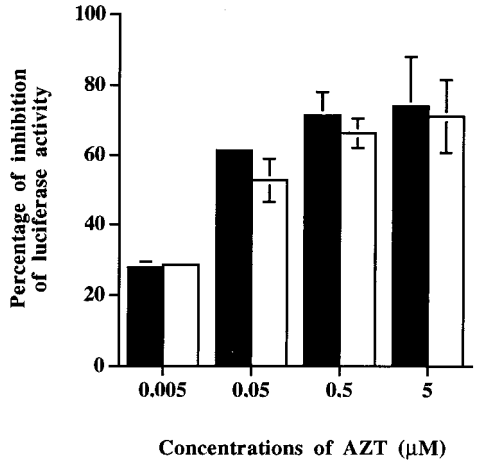

FIG. 6. AZT pretreatment leads to a comparable decrease of HIV-1 LTR-driven luciferase activity following infection with virions carrying or not carrying host-derived ICAM-1. 1G5 cells were pretreated with increasing concentrations of AZT before infection with 10 ng of p24 from each virus stock (ICAM-1/POS [■] and ICAM-1/NEG [□]). After initial infection, cells were maintained for 48 h with identical drug pressure before the measurement of luciferase activity. Results are percentages of inhibition relative to untreated cells and are the means ± SEM for samples run in triplicate.

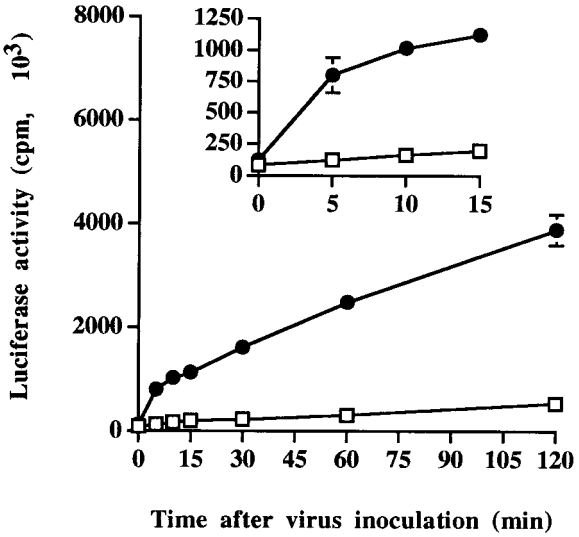

FIG. 8. Kinetics of infection with ICAM-1-positive and -negative viruses. 1G5 cells were inoculated with 10 ng of p24 from either ICAM-1-bearing virions (ICAM-1/POS [●]) or ICAM-1-free progeny viruses (ICAM-1/NEG [□]). The process of virus infection was terminated by adding, at specific time points, plasma-pooled purified human IgG from HIV-1-infected subjects. Cells were then washed twice with PBS to remove unadsorbed virions and were incubated for 24 h before addition of 1 μM AZT and 30 μM ddI. After a 24-h incubation, the cells were lysed and luciferase activity was monitored. Results are the means ± SEM for triplicate samples.

on HIV-1 increases virus infectivity. The results shown in Fig. 6 led us to suggest that virally acquired host cell membrane ICAM-1 exerts its effect on the virus infection process early, before reverse transcription. To test this possibility, we performed a time-course infection experiment by adding neutralizing antibodies to block the infection process at various time points following virus inoculation. Luciferase activity was found to increase almost linearly over the 120-min time period of the experiment in 1G5 cells infected with either ICAM-1-bearing or ICAM-1-negative virions, but the slope was much more abrupt when infection was achieved with ICAM-1/POS virus particles (Fig. 8). Thus, the kinetics of virus infection are more rapid with ICAM-1-bearing virions compared to infection with progeny viruses devoid of host-derived ICAM-1. For example, infection of 1G5 cells with ICAM-1/POS progeny viruses induced a 6.6-fold increase in HIV-1 LTR-driven luciferase activity after only 5 min of infection, compared to the reporter gene activity seen in cells inoculated with ICAM-1-negative virions (insert of Fig. 8). This 6.6-fold increase in HIV-1 LTR-dependent luciferase activity detected at 5 min postinfection was comparable to the enhancement of reporter gene activity after virus inoculation for 120 min (7.3-fold). Altogether, the results from these studies clearly indicate that virally embedded host cell membrane ICAM-1 glycoproteins positively affect the first step(s) in the infection of permissive cells, probably the adsorption-penetration steps.

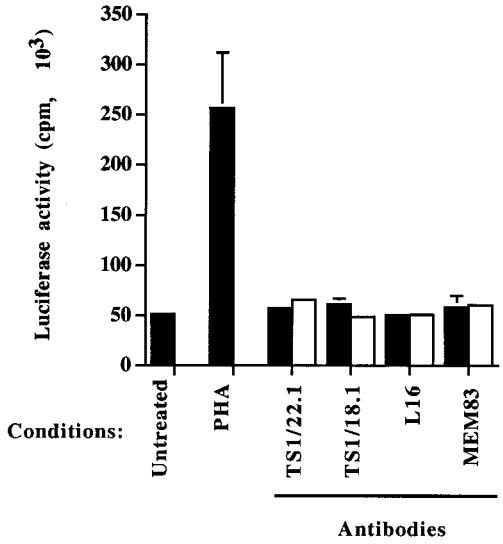

FIG. 7. HIV-1 LTR-dependent luciferase activity is not modulated by cross-linking of either LFA-1 alone or LFA-1 and CD4 simultaneously. 1G5 cells were preincubated with indicated anti-LFA-1 antibodies only (■) or with a combination of anti-LFA-1 and anti-CD4 antibodies (□) prior to incubation in 96-wells plate coated with GAM antibodies. After a 48-h incubation, cells were lysed and luciferase activity was monitored. Results are the means ± SEM for triplicate samples.

Enhancement of HIV-1 infection conferred by virion-bound cellular ICAM-1 is also seen in cells of the monocytoid lineage. Finally, we wanted to test if the enhancement of HIV-1 infection seen in 1G5 T-lymphoid cells can also be observed in monocytoid cell lines. To achieve such a goal, ICAM-1/NEG and ICAM-1/POS virions were used to infect BF24, a derivative of the THP-1 cell line that carries a stably integrated construct containing the CAT reporter gene under the control of the HIV-1 LTR promoter. It should be noted that BF24 cells are expressing LFA-1 on their surface (data not shown). The results from Fig. 9 indicate that the presence of host-derived ICAM-1 glycoproteins on HIV-1 also lead to an increase of virus infection in monocytoid cells. Enhancement of HIV-1 infectivity was abolished by pretreating ICAM-1-bearing progeny viruses with anti-ICAM-1 antibodies prior to infection, which is in agreement with our previous data with 1G5 T-lymphoid cells.

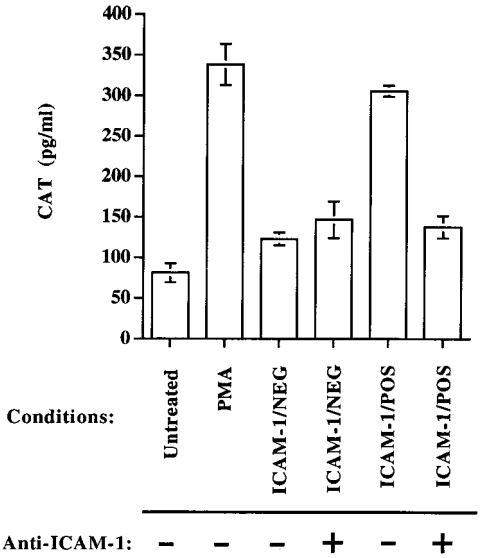

FIG. 9. Virally incorporated cellular ICAM-1 can also increase HIV-1 infectivity in monocytoid cells. BF24 monocytoid cells were infected with 25 ng of p24 from either ICAM-1-free progeny viruses (ICAM-1/NEG) or ICAM-1-bearing virions (ICAM-1/POS). At 24 h postinfection, 1 μM AZT and 30 μM ddI were added and, 24 h later, cells were lysed and CAT production was evaluated with a commercial ELISA kit (Boehringer). In this experiment, phorbol myristic acid (PMA at 20 ng/ml [Sigma]) was used as a positive control for HIV-1 LTR-mediated transcriptional activation of the luciferase reporter gene. Results are the means ± SEM for samples carried out in triplicate.

DISCUSSION

Adhesion molecules are responsible for the recognition and attachment of cells during various processes. They help to surpass the repulsive forces between the negative charges of cell membranes. ICAM-1 is found on most of the hematopoietic cells and on numerous nonhematopoietic cells, and its expression is increased upon the activation of T and B cells (19). Its counterreceptor, LFA-1, is constitutively expressed on lymphocytes, granulocytes, monocytes, and macrophages (54). The binding of LFA-1 to ICAM-1 is certainly the most important type of interaction between adhesion molecules for cellular functions. The LFA-1–ICAM-1 association enables cells of the immune system to interact with each other by increasing the avidity of intercellular contact and by allowing an adequate lapse of time for signal exchange to take place (54). The contribution of adhesion molecules to cell-to-cell transmission of HIV-1 has been the subject of several studies (23, 28, 30, 48), while the role of HIV-1-incorporated cellular adhesion molecules in the process of cell-free infection is just beginning to receive some attention (40, 51). The aim of the present study was to shed light on the participation of virally embedded adhesion molecule ICAM-1 in the process of infection by cell-free HIV-1. We developed transient expression and viral infection systems that allowed us to assess the functionality of the virion-embedded cellular ICAM-1 and to achieve quantitative measurements of HIV-1 infection.

We have determined that the presence of virally incorporated ICAM-1 was associated with a marked increase in HIV-1 LTR-driven luciferase activity following infection of 1G5, a sensitive T lymphoid reporter cell line useful for quantitative measurements of HIV-1 infection. Indeed, infection of 1G5 cells with HIV-1_{NL4-3} ICAM-1/POS particles resulted in a 4.6- to 9.8-fold increase in HIV-1 LTR-mediated luciferase activity compared with inoculation with HIV-1_{NL4-3} ICAM-1/NEG particles. Two distinct observations led us to conclude that the increase in HIV-1 infectivity is not associated with integrin-mediated signaling events. Firstly, HIV-1 LTR-driven luciferase activity in 1G5 cells was not up-regulated by cross-linking surface LFA-1 only, the physiological counterreceptor of ICAM-1, or LFA-1 and CD4 simultaneously (Fig. 7). Secondly, pretreatment of 1G5 cells with AZT resulted in a similar percentage of reduction of HIV-1 LTR-driven luciferase activity following virus inoculation with virions bearing or not bearing cellular ICAM-1 (Fig. 6). The finding that pretreatment with AZT does not completely block virus infection still has to be explained, but we believe that the residual 26 to 29% of HIV-1 LTR-driven luciferase activity could be a cell line-specific phenomenon. A partial inhibition of virus infection with AZT has been described previously by Aguilar-Cordova et al., who reported a 25% residual HIV-1 LTR-dependent luciferase activity following treatment of 1G5 cells with high concentrations of AZT prior to infection with different retroviruses, including HIV-1 (2).

Our data clearly indicate that, when similar amounts of ICAM-1/POS and ICAM-1/NEG virions standardized for their p24 contents are used, ICAM-1-bearing virions are more infectious than their ICAM-1-free counterparts. The net advantage in terms of infectivity conferred by host-derived ICAM-1 implies that ICAM-1 retains its natural function even when it is located on the virion surface. The demonstration that pretreatment of ICAM-1-bearing virions with RR1/1.1.1 can abolish the enhancement of HIV-1 infectivity (Fig. 3 and 9) is further evidence of the functionality of virally incorporated cellular ICAM-1, since this anti-ICAM-1 antibody can block ICAM-1–LFA-1-dependent cell-cell interactions (50). Moreover, the fact that pretreatment of 1G5 cells with an anti-LFA-1 antibody (MEM30) before infection can also eliminate the increase in HIV-1 infectivity of the ICAM-1/POS virions represents another demonstration that the interaction between virion-bound ICAM-1 and cell surface LFA-1 is responsible for the infectivity advantage (Fig. 5). To the best of our knowledge, this is the first direct demonstration that virally embedded host surface membrane ICAM-1 glycoproteins are biologically functional. The results from our experiments are of prime importance, considering that clinical isolates of HIV-1 grown on mitogen-stimulated primary mononuclear cells have been shown to incorporate host-derived ICAM-1 glycoproteins (9).

It has been previously postulated that the presence of host-derived adhesion molecules may play an important role as accessory molecules in the process of HIV-1 infection (47). An increase in virus infectivity was reported by Castiletti et al. for HIV-1 particles harvested from IFN-γ-treated U937 cells (11). The up-regulation of HIV-1 infection seen in this report could not be attributed exclusively to host-derived ICAM-1 incorporated on progeny viruses, since several cell surface molecules are modulated by IFN-γ. However, one report has previously provided evidence for the importance of virally embedded cellular ICAM-1 molecule in the viral infection process by demonstrating that pretreatment of monocytes with antibodies against LFA-1 and LFA-3 can block HIV-1 infection (33). The results from this study confirm our observations indicating that virion-embedded cellular ICAM-1 glycoproteins positively affect infection with cell-free HIV-1 particles.

Our studies indicate that the mechanism by which virally embedded cellular ICAM-1 glycoproteins can upregulate HIV-1 infectivity is probably through an increase of the overall virus-binding avidity, thereby favoring early steps in the virus replicative cycle. Recently, it was demonstrated that monomeric and multimeric forms of ICAM-1 had affinity constants for LFA-1 of 190 and 4 nM, respectively (57). The high disso-

ciation constant determined for binding of multimeric ICAM-1 to LFA-1 is similar to the binding constant for the gp120-CD4 interaction (K_d = 4 nM) (37). Based on the marked enhancement of virus infectivity detected in our studies, we can presume that cell-derived ICAM-1 on the virion surface is probably under its multimeric form. These additional strong virus-cell interactions may allow the virus to overpass more efficiently the electrostatic repulsion normally present between viral and cellular surfaces. The kinetics of virus infection further support the idea that ICAM-1-bearing virions possess a greater affinity and/or avidity for HIV-1-susceptible cells, since they enter cells more rapidly than progeny virus devoid of host-derived ICAM-1.

The acquisition of host-derived ICAM-1 on HIV-1 can positively affect HIV-1 infectivity, as demonstrated by our study, but could also potentially influence other aspects of the virus biology. For example, the sensitivity to antibody-mediated neutralization is another aspect of HIV-1 that could be modulated by the incorporation of host-derived ICAM-1. This is reminiscent of a previous report showing that addition of anti-LFA-1 monoclonal antibodies to plasma originating from HIV-1-infected subjects can enhance their neutralizing capacity (27). Berman and Nakamura have shown that interactions between ICAM-1 and LFA-1 can reduce the syncytium-inhibiting activity of virus-neutralizing monoclonal antibodies and soluble CD4 in a virus-free cell fusion system (7). They proposed that the auxiliary adhesion molecules could enhance the process of fusion by various mechanisms, such as increasing the probability of intercellular contact between potential gp160- and CD4-bearing fusion partners or enhancing the effective avidity between gp120 and CD4 on the surface of infected cells. Our results suggest that virion-bound ICAM-1 glycoproteins could play a similar role in the case of cell-free HIV-1 infection. It has also been proposed that the increased resistance of viruses grown on primary peripheral blood mononuclear cells, compared to virions grown on continuous cell lines, to neutralization by soluble CD4 and antibodies was due in part to levels of ICAM-1 surface expression on activated peripheral blood mononuclear cells higher than those on T-lymphoid cell lines (7, 27).

Previous studies aimed at studying early steps of the virus life cycle indicated that HIV-1 infection is more complex than a simple gp120-CD4 interaction. The recent discovery of the fusogenic coreceptors (3, 13, 15, 17, 18, 25) and the growing evidence of an important role for virally incorporated cell surface membrane constituents are urging us to revise our conception of the HIV-1 infection process. The results from our experiments lead us to propose that infection with HIV-1 resembles cell-to-cell contacts in which strong interactions such as gp120-CD4 and LFA-1–ICAM-1 interactions would favor a rapid adhesion of the virus to the target cell and would help to maintain the virus-cell conjugate long enough for the fusion to occur. All of the studies involving virally acquired cell surface molecules will surely be crucial in reaching a better understanding of the process of infection of target cells in vivo by HIV-1 particles.

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J.-F. Fortin and R. Cantin contributed equally to this work.

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